UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/568,745	02/21/2006	Mitsuko Ideno	1422-0709PUS1	6655

2292 7590 09/07/2011
BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747

EXAMINER	
SKELDING, ZACHARY S	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
09/07/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/568,745	IDENO ET AL.	
	Examiner	Art Unit	
	ZACHARY SKELDING	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *6-20-11 and 7-11-11*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-4,6-9 and 13-24* is/are pending in the application.

5a) Of the above claim(s) *14,17-19 and 22-24* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-4,6-9,13,15,16,20 and 21* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7-11-11*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment and remarks filed June 20, 2011 are acknowledged.

   Claims 1-4, 6-9 and 13-24 are pending.

   Claims 1-4, 6-9, 13, 15, 16, 20 and 21 are under examination wherein the elected species of fibronectin fragment is SEQ ID NO: 13 and wherein the method includes a step of diluting a cell culture solution.

   Claims 14, 17-19 and 22-24 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to non-elected Group or species of invention.

   The rejection under 35 U.S.C. § 103(a) in view of Sagawa, Johnson etc. has been withdrawn in view of applicant's claim amendments. In particular, amending the claims to recite "(a) culturing PBMC wherein said PBMC are capable of differentiating into cytotoxic lymphocytes…wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface…and wherein the method, optionally, further comprises, maintaining or expanding the cytotoxic lymphocytes obtained in step (a) with a medium..." limits the claimed method by excluding the presence of an antigen-presenting cell comprising antigenic peptide on its surface; however, that said, the claim as amended is indefinite for the reasons given below.

   The rejection under 35 U.S.C. § 112, 2nd paragraph has been withdrawn in view of applicant's claim amendments.

   That said, a new grounds of rejection under 35 U.S.C. § 112, 2nd paragraph is put forth below in view of applicant's claim amendments.

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

   > The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 1-4, 6-9, 13, 15, 16, 20 and 21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

   Claim 1, and dependent claims thereof, recites: "A method for preparing a cytotoxic lymphocyte which method comprises (a) culturing peripheral blood mononuclear cells wherein said peripheral blood mononuclear cells are capable of differentiating into cytotoxic lymphocytes with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences

shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2, wherein said fibronectin fragment comprises a cell adhesion activity and/or a heparin binding activity, wherein a cytotoxic activity is enhanced as compared to a cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of the recombinant fibronectin fragment, wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface, and wherein the method, optionally, further comprises, maintaining or expanding the cytotoxic lymphocytes by culturing the cytotoxic lymphocytes obtained in step a) with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2.

The meaning of the instant claims would be unclear to the skilled artisan for several reasons.

First, it would be unclear to the skilled artisan from the language of step (a) ("culturing peripheral blood mononuclear cells wherein said peripheral blood mononuclear cells are capable of differentiating into cytotoxic lymphocytes with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment…") if the step is positively reciting the PBMC must be cultured "in a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment…" or if this phrase ("in a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment…") is merely a description of the properties of the PBMC of step (a), and that step (a) while specifying "culturing PBMC," does not place any limits on how these PBMC must be cultured.

Second, the claim includes the limitation "wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface." However, PBMC comprise antigen-presenting cells comprising antigenic peptides on their surface, e.g., B cells and monocytes. Thus, the effect of this limitation on the metes and bounds of the claimed invention would be unclear to the skilled artisan. For example, does this limitation mean the skilled artisan must interpret "peripheral mononuclear cells" to refer to only a certain type of blood cell that is not "an antigen-presenting cell comprising antigenic peptide on its surface," such as NK cells?

In this regard it is noted that the phrase "peripheral mononuclear cells" is often understood by the skilled artisan based on the context in which it is used. For example, B-cells, T-cells, NK cells and monocytes may *each* be described as "peripheral mononuclear cells" and referred to as such. On the other hand, the skilled artisan may describe, e.g., using leukapheresis to harvest bulk "peripheral mononuclear cells" from blood, in which case the phrase "peripheral mononuclear cells" is understood to refer to the collection of B-cells, T-cells, NK cells and monocytes found in blood.

In conclusion, the metes and bounds of the claimed method would be unclear to the skilled artisan. Thus, it would not be possible for the skilled artisan to ascertain what activities would or would not infringe upon the instant claims should they become patented.

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1-4, 6-9, 13, 15, 16, 20 and 21 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for

A method for preparing a population of cytotoxic lymphocytes comprising

obtaining peripheral mononuclear cells which have an ability to differentiate into cytotoxic lymphocytes,

culturing said peripheral mononuclear cells in a medium containing added IL-2 and further containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume,

wherein said culturing is performed in the presence of a recombinant fibronectin fragment which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing, wherein said fibronectin fragment comprises a cell adhesion activity and/or a heparin binding activity,

and wherein a cytotoxic activity is enhanced or a high cytotoxic activity is maintained as compared to a cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of the recombinant fibronectin fragment.

does not reasonably provide enablement for a method for preparing a cytotoxic lymphocyte which method comprises (a) culturing peripheral blood mononuclear cells wherein said peripheral blood mononuclear cells are capable of differentiating into cytotoxic lymphocytes with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2, wherein said fibronectin fragment comprises a cell adhesion activity and/or a heparin binding activity, wherein a cytotoxic activity is enhanced as compared to a cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of the recombinant fibronectin fragment, wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface, and wherein the method, optionally, further comprises, maintaining or expanding the cytotoxic lymphocytes by culturing the cytotoxic lymphocytes obtained in step a) with a

medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2.

Applicant argues the claims as amended address the prior enablement rejection.

This is not found convincing because, as put forth above, when the instant claims are given their broadest reasonable interpretation consistent with the instant specification they encompass in their breadth a method for preparing cytotoxic lymphocytes comprising a step (a) which explicitly specifies "culturing PBMC" having certain properties ("capable of differentiating into cytotoxic lymphocytes with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment") without placing any limits on how these PBMC will be cultured.

Therefore, the outstanding enablement rejection is maintained for the reasons of record.

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1-4, 6-9, 13, 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Frederick Darfler (WO 88/02774) in view of Ochoa et al. (Cancer Res. 1989 Feb 15;49(4):963-8), Cardarelli et al. (Cell Immunol. 1991 Jun;135(1):105-17) and Taguchi et al. (U.S. Patent 5,198,423), and further in view of Whiteside et al. ("Current Protocols in Immunology," 1996, Supp. 17, Unit 7.7, pages 7.7.1-7.7.11, submitted on IDS filed July 11, 2011), essentially for the reasons of record as put forth in the Office Action mailed March 18, 2011 and as described further below.

Applicant argues the claimed methods are non-obvious because the presence of fibronectin has an effect on PBMCs that could not have been expected by the ordinary artisan at the time of the invention (see remarks page 10-11).

Applicant's argument is not found convincing because, first, with respect to the effect of recombinant fibronectin on cell expansion, it would not have been surprising to one of ordinary skill in the art that fibronectin increases PMBC expansion based on the teachings of Cardarelli as put forth in the prior Office Action.

Moreover, applicant's allegation that the ordinary artisan could not have reasonably expected substitution of recombinant fibronectin for the native fibronectin of Cardarelli to have the same effect is also not convincing because as put forth in the prior Office Action, one of ordinary skill in the art knew that the parts of native fibronectin responsible for its effect on T cells as taught Cardarelli, i.e., the RGD and EILDV sequences, were present in the recombinant fibronectin fragment comprising SEQ ID NO: 13 of Taguchi.

With respect to applicant's argument that the induction of CD25 would not have suggested to one of ordinary skill in the art that cytotoxicity is enhanced, this is not found convincing because it is contrary to the knowledge of one of ordinary skill in the art as exemplified by the teachings of, e.g., Ochoa and Darfler, that IL-2 is essential for activating PBMC cytotoxicity. Moreover, the mechanism by which IL-2 induces the PBMC cytotoxicity as described by Whiteside explains how induction of CD25 expression is closely linked with the induction of PBMC cytotoxicity (see pages 8 and 9, "LAK activation").

With respect to applicant's argument that one of ordinary skill in the art could not have expected the expansion of LAK cells shown in Table 43 / example 43 of the instant specification, which describes the expansion of PBMC in serum-free AIM-V medium + anti-CD3 + IL-2 ± CH296 fibronectin, this is not found convincing for several reasons.

First, applicant is not comparing the claimed invention with the closest prior art teachings of Darfler, e.g. , comparing the production cytotoxic T cells from PBMC using serum-free Darfler medium + IL-2 vs. serum-free Darfler medium + IL-2 + CH296 fibronectin.

Secondly, even if the results of Table 43 could be argued to be viewed as surprising by one of ordinary skill in the art, this would not be sufficient to overcome the instant prima facie case of obviousness given:

(1) the strong motivation in the art for the ordinary artisan to generate large numbers of lymphokine activated cytotoxic lymphocytes from a patient's PBMC and

(2) the combined reference teachings as put forth in the prior Office Action and further augmented by the teaching of Whiteside above which strongly suggest the skilled artisan would have a reasonable expectation that peripheral mononuclear cells expanded in the presence of anti-CD3 + fibronectin + IL-2 would not only be expanded to large numbers but would also be fully sensitive to the LAK-inducing effects of IL-2 via binding of IL-2 to CD25.

8. Claims 20 and 21 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Frederick Darfler (WO 88/02774) in view of Ochoa et al. (Cancer Res. 1989 Feb 15;49(4):963-8), Cardarelli et al. (Cell Immunol. 1991 Jun;135(1):105-17), Taguchi et al. (U.S. Patent 5,198,423) and Whiteside et al. ("Current Protocols in Immunology," 1996, Supp. 17, Unit 7.7, pages 7.7.1-7.7.11) as applied to claims 1-4, 6-9, 13, 15 and 16 above, and further in

view of Chen et al. (J Immunol. 1994 Oct 15;153(8):3630-8), essentially for the reasons of record as put forth in the Office Action mailed March 18, 2011.

Applicant argues Chen teaches the transduction of PKC genes into cytotoxic T cells, which is not specified by the instant claims, and applicant further argues one of ordinary skill in the art would not have been motivated to combine the teachings of Chen with Darfler, Ochoa, Cardarelli and Taguchi.

Applicant's arguments have been considered, but have not been found convincing, essentially for the reasons of record as put forth in the Office Action mailed March 18, 2011.

Applicant's argument is not convincing because the instant claims use the open-ended transitional language "comprising" and thus encompass the transduction of PKC genes into cytotoxic T cells contained within PBMC. Moreover, applicant argument about a lack of motivation to combine is conclusory and does not convincingly addressed the motivation to combine put forth in the prior Office Action.

9. Claims 1-4, 6-9, 13, 15, 16, 20 and 21 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Parker et al. (Hum Gene Ther. 2000 Nov 20;11(17):2377-87) in view of Bagnis et al. (WO 99/05301) as evidenced by Bagnis et al. (6,287,864) which is the English language U.S. National Stage entry of WO 99/05301, and as further evidenced by the Gibco/Invitrogen Publication "A Guide to Serum-Free Cell Culture," 2003, pages 1-7 as well as Kaldjian et al. J Immunol Methods. 1992 Mar 4;147(2):189-95, essentially for the reasons of record as put forth in the Office Action mailed March 18, 2011.

Applicant argues "Parker employs serum-containing medium in a gene transfer process. Bagnis also applies recombinant fibronectin fragment in a gene transfer process, but not for cell culture. In this light, the claimed methods, Parker and Bagnis are distinguishable. Furthermore, in regard to cell culturing, after day 5 Parker teaches that T-cells are cultured without a recombinant fibronectin fragment. In contrast, the claimed methods use a recombinant fibronectin fragment when 'culturing' peripheral blood mononuclear cells in order to induce cytotoxic T lymphocytes, which is one of the features of the present invention. The claimed methods do not use recombinant fibronectin fragment in a gene transfer process."

Applicant's arguments have been considered, but have not been found convincing, essentially for the reasons of record as put forth in the Office Action mailed March 18, 2011.

Applicant's argument is not convincing because, first as put forth in the prior Office Action, Parker teaches a method for preparing cytotoxic lymphocytes comprising culturing peripheral mononuclear cells in AIM V medium which is serum free. With regard to applicant's allegation about the teachings of Bagnis, this is not convincing because it is unclear how one of ordinary skill in the art is not "culturing PBMC" when transducing PBMC with the MOv-γ gene as described by Parker. Indeed, this notion is reflected in the teachings of Bagnis.

For example, at col. 2, 3rd paragraph (emphasis added), Bagnis states "In the gene transfer methods using the retrovirus as described above, infection with the retrovirus (i.e., gene transfer) ***occurs when the target cells are cultured in a medium containing the retrovirus***. A medium containing a serum from an animal (in many cases, fetal calf serum (FCS)) is used in this step. Since the serum contains constituents that can serve as nutrients for cells and various growth factors, it is believed that the serum is highly effective for maintaining cells in vitro."

Similarly, at col. 7, 1st paragraph (emphasis added), Bagnis states "By infecting cells with a retrovirus in a serum-free medium in the presence of an effective amount of the fibronectin, the fibronectin fragment or the mixture thereof, cells with transferred genes can be efficiently obtained. The fibronectin, the fibronectin fragment or the mixture thereof may be ***immobilized on the surface of the culture vessel*** used for the infection with the retrovirus, for example. The infection with the retrovirus can be performed according to a conventional method, for example, by incubation at 37.degree. C. in 5% CO.sub.2. The conditions and the incubation time may suitably changed depending on the target cells or the objects."

With respect to the teachings of Parker that the T cells are cultured without recombinant fibronectin after day 5 which is allegedly distinct from the claimed invention, this is not found convincing because applicant appears to be arguing a limitation not claimed in that the instant claims do not specify a time period over which the "culturing" step must occur.

With respect to applicants argument that the claimed methods do not use recombinant fibronectin fragment in a gene transfer process, this is not found convincing in view of dependent claims 20-21 and the open-ended transitional language used in the instant claims.

10. Claims 1-4, 6-9, 13, 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sagawa et al. ("Ko-CD3 Kotai to RetroNectin® o Kumiawaseru Koto ni yoru LAK Saibo Invu Ryoho no Kairyo," Dai 62 Kai Annual Meeting of the Japan Cancer Association Kiji, 2003, page 438, English Translation, cited on the IDS filed July 11, 2011), in view of Ochoa et al. (Cancer Res. 1989 Feb 15;49(4):963-8), Whiteside et al. ("Current Protocols in Immunology," 1996, Supp. 17, Unit 7.7, pages 7.7.1-7.7.11), Cardarelli et al. (Cell Immunol. 1991 Jun;135(1):105-17) and Taguchi et al. (U.S. Patent 5,198,423).

Sagawa teaches "As one of immunotherapies for cancers, a transfusion therapy of LAK cells activated with an anti-CD3 antibody and IL-2 has been performed. In this therapy, it is crucial that a large amount of LAK cells are obtained from peripheral blood mononuclear cells (PBMC) in a short time period." Sagawa further teaches a recombinant human fibronectin fragment known as RetroNectin® (which is identical to the elected species of fibronectin, SEQ ID NO: 13), increases the expansion fold of LAK cells cultured in the presence of anti-CD3 antibody. (see entire document).

Sagawa differs from the claimed invention in that it is not clear if Sagawa is explicitly teaching culturing PBMC in the presence of IL-2, anti-CD3 and RetroNectin®, or just anti-CD3 and RetroNectin®. Moreover, Sagawa does not explicitly teach the use of immobilized RetroNectin®.

With respect to culturing PBMC in the presence of IL-2, anti-CD3 and RetroNectin®, or just anti-CD3 and RetroNectin®, even though the English language translation of Sagawa is not clear in this regard, it would have been obvious to one of ordinary skill in the art that the optimal production of LAK cells from PBMC requires the presence of IL-2 given the teachings of Ochoa at page 967 col. bridging paragraph and Whiteside at pages 8-9.

Furthermore, with respect to using immobilized RetroNectin®, Cardarelli teaches the addition of immobilized fibronectin and IL-2 to PBMC cultures stimulated with anti-CD3 in the presence of serum-free media enhances proliferation and IL-2R expression of T lymphocytes (see Fig. 1 and Tables 2 and 3, in particular). Cardarelli further teaches that the regions of fibronectin responsible for its activity on T cells are the RGD cell binding domain and the EILDV amino acid sequence (see page 115, in particular). Cardarelli also teaches that the cells can be cultured at a concentration of 10^5 cells/well of a microtiter plate (i.e. at a concentration between 1 and 5 x 10^5 cells/ml).

Taguchi teaches a biologically active recombinant fibronectin fragment comprising SEQ ID NO: 13 (see Example 4). Said fragments comprise the RGD and EILDV sequences responsible for the activity of fibronectin on T cells as taught Cardarelli. The '423 patent also teaches that the recombinant fibronectin is advantageous compared to natural fibronectin, which is limited in supply, costly to produce, and potentially contaminated with bacteria and viruses (see column 1 in particular).

Therefore, given the reference teachings it would have been obvious to one of ordinary skill in the art to generate LAK cells by culturing PBMC in the presence of IL-2, anti-CD3 and RetroNectin®. One of ordinary skill in the art would have been motivated to do so because as taught by Sagawa culturing PBMC with anti-CD3 and RetroNectin® increases the expansion fold and CD25 expression levels of LAK cells. As would be obvious to one of ordinary skill in the art, a method of generating large amounts of activated, cytotoxic LAK cells is advantageous for adoptive immunotherapy.

Moreover, as to the use of the immobilized fibronectin fragment SEQ ID NO: 13 in the method for preparing cytotoxic lymphocytes, the ordinary artisan would have been motivated to use immobilized fibronectin because using such a format is effective according to the teaching of Cardarelli. Also, because fibronectin is an extracellular matrix protein one of ordinary skill in the art would consider immobilization of fibronectin to the tissue culture solid phase a better mimic of its physiologic state than in solution.

Furthermore, it would have been obvious to one of ordinary skill in the art, and one of ordinary skill in the art would have been motivated to substitute the recombinant fibronectin

fragment of Example 4 taught by Taguchi for the purified human fibronectin taught by Cardarelli since Taguchi teaches recombinant fibronectin is advantageous compared to natural fibronectin, which is limited in supply, costly to produce, and potentially contaminated with bacteria and viruses. Moreover, one of ordinary skill in the art would have had a reasonable expectation of successfully substituting the recombinant fibronectin fragment of Taguchi for purified human fibronectin because recombinant fibronectin is a biologically active fragment comprising the sequences taught by Cardarelli as being important for T cell simulation.

As to claims reciting steps of diluting or exchanging the cell culture medium, or exchanging the cell culture equipment, Ochoa teaches conditions wherein diluting or exchanging the cell culture medium is a useful step, such as in the instance of cell over-crowding, and these techniques are well known to one of ordinary skill in the art (see Ochoa at page 963, materials and methods.

In conclusion, given the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in arriving at the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

11. No claims are allowed.

12. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Moreover, applicant's submission of an information disclosure statement under 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p) on July 11, 2011 prompted the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 609.04(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZACHARY SKELDING whose telephone number is (571)272-9033. The examiner can normally be reached on Monday - Friday 8:00 a.m. - 5:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Zachary Skelding/
Primary Examiner, Art Unit 1644